Topic 33 – Gene therapy, cell therapy

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0401

Electrospun collagen scaffolds for the cardiac graft of cardiomyocytes derived from human pluripotent stem cells

Solène Boitard (1), Pierre Joanne (1), Maria Kitsara (2), Mathieu Pernot (3), Patricia Forest (4), Valérie Vanneaux (5), Jérôme Larghero (5), Philippe Menasché (6), Yong Chen (2), Onnik Agbulut (1)
(1) Université Pierre et Marie Curie, Biological Adaptation and Ageing (B2A) – UMR8256, Paris, France – (2) Ecole Normale Supérieure, Paris, France – (3) Institut Langevin ESPCI ParisTech, Paris, France – (4) Biom'Up, Saint-Priest, France – (5) APHP-Hôpital Saint-Louis, Laboratoire de thérapie cellulaire, Paris, France – (6) APHP-Hôpital Européen Georges Pompidou (HEGP), INSERM U633, Paris, France

Collagen, the most abundant component of extracellular matrix, is a molecule of choice for the development of cellular biomaterials, because it can interact with most cell types and can facilitate their adhesion and growth. In this study, electrospun nanofibrous collagen patches was used to provide a biocompatible physical support for the graft of human pluripotent stem cells derived cardiomyocytes (hPCS-CM) for the treatment of the failing heart.

Different types of clinically approved collagen were studied for their ability to form high quality uniform fibers by electrospinning using a benign solvent system based on ethanol, water and salts. Atelocollagen – that contains 95% of type I and 5% of type III – exhibited the best performance. After appropriate crosslinking using cytocompatible agents based on citric acid (concentrated to 5% or 10%), the collagen was placed to special holders for cell culture.

The electrospun collagen patches were used for culture of newborn rats cardiomyocytes, showing regular beating after 3 days of culture. They already have been successfully used for the culture of hPSC-CM. Finally, the electrospun collagen patches have been implanted in mice with dilated cardiomyopathy and have exhibited excellent biocompatibility. Cardiac function measured by echocardiography before and after the graft, histological, and molecular studies showed no detrimental effects of the collagen scaffold. Our next target is the implantation of hPSC-CM-seeded scaffold in mice with dilated cardiomyopathy.

0202

Adult cardiomyocytes proliferation blockage by nuclear ephrin-B1: involvement of the PI3Kγ pathway

Marie Cauquil (1), Céline Mias (1), Marie-Hélène Séguélas (1), Céline Guilbeau-Frugier (2), Adrien Lupieri (1), Muriel Laffargue (1), Atul Pathak (3), Jean-Michel Sénard (3), Céline Galés (1)
(1) CHU Toulouse Rangueil, I2MC, Toulouse, France – (2) CHU Toulouse, Histopathologie, Toulouse, France – (3) CHU Toulouse, Pharmacologie clinique, Toulouse, France

The possibility to reboot the proliferation of adult resident cardiomyocytes (CMs) has emerged as a promising avenue in cardiac regenerative medicine. For that purpose, there is an urgent need for identifying the molecular mechanisms involved in the natural blockage of adult CMs proliferation. Recently, we recently demonstrated that ephrin-B1 expression in the CMs nuclei acts as a specific inhibitor of the adult CMs proliferation (unpublished data). Here, we have explored the specific role of the PI3Kγ in the nuclearization of ephrin-B1.

Fluorescent immunostaining showed a specific loss of nuclear ephrin-B1 in p110-γ KO but not in KI mice, suggesting a specific role of the PKA-anchoring function of the p110-γ catalytic subunit of PI3Kγ in ephrin-B1 nuclearization. Interestingly, p110-γ KO mice demonstrated a significant increase of mononucleated CMs (19,1 ± 1,1 % vs 13,3 ± 1,0 % in WT) correlated to a decrease in binucleated CMs (70,9 ± 1,9 % vs 79,7 ±1,0 % in WT) possibly indicative of a proliferative potential. In agreement with a proliferative potential, we measured a higher level of replicative-BrdU+ CMs in KO PI3Kγ mice (WT: 6,23±2,2 % ; KO: 19,3±4,9 %; KI: 3,0±0,8 %), but also a higher CMs density in cardiac tissue from 8 month old mice (WT: 2633±79,4cm/mm²; KO: 3208±155,6CM/mm²; KI: 2659,7±99,3CM/mm²) mosy likely reflecting CMs hyperplasia. To confirm the involvement of a cAMP/PKA pathway in ephrin-B1 nuclearization, we studied a neonatal cardiomyoblast cell line (H9C2) naturally expressing a nuclear ephrin-B1 pool. We showed that H89 treatment prevented ephrin-B1 nuclearization while forskolin promoted nuclearization of a recombinant ephrin-B1-GFP.

These results demonstrated for the first time the specific role of the cAMP/PKA/PI3Kγ pathway in ephrin-B1 nuclearization in the CM. In the future, the accurate characterization of this pathway could allow identifying new and more specific targets for heart regenerative medicine strategies.

0413

Cardiac differentiation of human pluripotent stem cells: the first step toward cardiac tissue engineering and cell therapy

Pierre Joanne, Solène Boitard, Solenne Paiva, Onnik Agbulut
Université Pierre et Marie Curie, Unité B2A, UMR8256, Paris, France

Human pluripotent stem cells derived cardiomyoctyes are one the most promising cells for cardiac research, i.e. disease modeling, drug screening and cell therapy applications. Obtaining these cells is a critical step and has been the subject of many studies and publications. Recent advances in this field have shown that a sequential activation and inhibition of the Wnt pathway induces the differentiation of human pluripotent stem cells toward the cardiac lineage. This procedure can be achieved in a chemically-defined environment, and beating cells are obtained after 8 days.

Several parameters of this protocol could still be improved. For example, the small molecules used (Wnt activators and inhibitors) are not approved by the health authorities, preventing their use in clinical studies. Moreover, there are no standard procedures to recover the cardiomyocytes after the differentiation protocol. Finally, late-stage differentiation to a totally matured ventricular cardiomyocyte population has not been achieved for the moment. The immature cardiac cells obtained have several differences compared to adult cardiomyocytes. There are, for example, mismatches in cell size and shape, gap junction, alignment and calcium handling ability. Raise these technological barriers could lead to new perspectives in the use of these cells.

Our studies in this area have led us to develop innovative strategies to obtain, recover and to mature these cells. We have tested several FDA-approved drugs known to modulate the Wnt pathway in order to obtain a clinical-grade population cardiomyocytes. We also determined, using a combination of protease, the condition of recovery allowing the best survival rate and function of these cells. Finally, we improve the maturity of cardiomyocytes derived from pluripotent stem cells by tuning their micro-environment. Our results demonstrate the importance of the extracellular matrix composition and mechanical properties.

0190

Ephrin-B1 blocks adult cardiomyocyte proliferation and cardiac tissue regeneration

Celine Mias (1), Gael Genet (1), Celine Guilbeau-Frugier (1), Marie-Helene Seguelas (1), Denis Calise (2), Atul Pathak (1), Jean-Michel Senard (1), Celine Gales (1)
(1) CHU Toulouse Rangueil, INSERM UMR 1048, Toulouse, France – (2) CHU Toulouse Purpan, US006, Microchirurgie Expérimentale, Toulouse, France

The possibility to reboot the proliferation of adult resident cardiomyocytes (CM) has recently emerged as a new promising avenue in cardiac regenerative medicine. For that purpose, there is an urgent need for identifying the molecular mechanisms involved in the natural blockage of adult CM proliferation.

We recently identified ephrin-B1 as specific stabilizer of the CM rod-shape allowing the cardiac tissue cohesion. Interestingly, we found that ephrin-B1 knock-out mice (KO) compensate aging stress through a surprising CM hyperplasia, suggesting an atypical proliferation of adult CM. Cell cycle genes profiling performed by qRT-PCR showed that old KO CM significantly up-regulated genes involved in all cell cycle phases. Progression of CM throughout the cell cycle was confirmed by flow cytometry and revealed the presence of the replicative S-phase only in old KO CM. Proliferation was next

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directly assessed on isolated 2 months-old CM stimulated or not with neuregulin-1 growth factor. Remarkably, while this treatment resulted in 0.17% of BrdU uptake in WT CM, it increased up to 9.57 % in KO. KO CM also exhibited significant higher mitotic (pH3+) and cytokinesis (AuroraB+) events compared to WT. Thus, the capacity for adult KO CM to proliferate is not restricted to aged CM but more likely an intrinsic potential of young KO CM. We next assessed the proliferative capacity of 2 month-old KO mice in vivo using the apectomy model. Remarkably, while WT mice developed a classical healing process (fibrosis/inflammation), KO mice almost completely regenerated the apex as indicated by the presence of mitotic (BrdU+, pH3+, AuroraB+) CM and significant reduced fibrosis (50%) in the resected zone.

These results demonstrated that ephrin-B1 protein is a specific blocker of adult CM proliferation and its downregulation represents a huge interest for future therapeutic approaches in cardiac regenerative medicine.

0128

Sca-1 positive cells, but not c-kit positive cells, differentiate into mature cardiomyocytes after brain natriuretic peptide treatment

Stéphanie Rignault (1), Christelle Bielmann (1), Lucas Liaudet (2), Bernard Waeber (1), François Feihl (1), Nathalie Rosenblatt (1)
(1) CHU Vaudois, Physiopathologie, Lausanne, Suisse – (2) CHU Vaudois, Soins Intensifs Adultes, Lausanne, Suisse

The Brain Natriuretic Peptide (BNP) is a cardiac hormone, which promotes the recovery of cardiac function and the preservation of cardiac tissue in animal models of heart diseases. Its cardiac protective role in animals was attributed to fibrosis inhibition, as well as to reduction of cardiomyocyte apoptosis and hypertrophy. Recently, we demonstrated that BNP induces heart regeneration via the stimulation of cardiac precursor cell (CPC) proliferation and differentiation into mature cardiomyocytes.

The aim of our study was to identify which CPC's subset is able to respond to BNP stimulation.

Cardiac precursor cells identified as being Sca-1$^+$ Nkx2.5$^+$ or c-kit$^+$ Nkx2.5$^+$ cells, expressed in neonatal and adult hearts BNP's receptors (NPR-A and NPR-B), showing their ability to be activated by BNP treatment. Cell sorting experiments based on the expression of Sca-1 or c-kit were performed on non-myocyte cells isolated from neonatal wild-type hearts. Sca-1$^+$ and c-kit$^+$ cells were cultured up to 3 weeks with or without BNP in differentiating medium. Sca-1 positive cells, which contained few c-kit$^+$ cells, responded clearly to BNP stimulation by upregulating mRNA levels of genes coding for Nkx2.5, Mlc-2v, c-kit, Sca-1, beta and alpha MHC. Furthermore, higher number of Troponin I$^+$ cells was detected in BNP treated cells compared to untreated cells, suggesting that Sca-1$^+$ cells differentiated after BNP stimulation into mature cardiomyocytes. BNP treatment of c-kit$^+$ cells didn't induce the upregulation of mRNA coding for cardiomyocyte specific genes. However, we determined that c-kit positive cells spontaneously differentiated into mature cardiomyocytes during the 3 weeks of cell culture without BNP stimulation.

To determine which receptor is involved, Sca-1$^+$ cells, isolated from neonatal hearts of NPR-A or NPR-B deficient mice, were treated with BNP. The effects of BNP on wild type and NPR-A KO cells did not differ substantially. However, Sca-1$^+$ cells isolated from NPR-B deficient hearts couldn't respond anymore to BNP stimulation.

Thus, BNP specifically stimulates via NPR-B Sca-1$^+$ cell differentiation into cardiomyocytes. c-kit$^+$ cells display clearly a cardiogenic potential which is BNP independent.

0129

Adult human mononuclear clones isolated from peripheral blood can differentiate into immature cardiomyocytes

Suzanne Badoux (1), Stéphanie Rignault (1), Christelle Bielmann (1), Fabien Cusin (1), Lucas Liaudet (2), Bernard Waeber (1), François Feihl (1), Nathalie Rosenblatt (1)
(1) CHU Vaudois, Physiopathologie, Lausanne, Suisse – (2) CHU Vaudois, Soins Intensifs Adultes, Lausanne, Suisse

Breakthroughs in stem cell biology and demonstrations of the heart's endogenous regenerative capacities have incited an intense race towards cardiac regeneration, i.e. the replacement of lost myocardium after myocardial infarction. In preclinical trials, cell transplantation therapies, using adult human multipotent stem cells (e. g, hematopoietic stem cells) into infarcted myocardium has shown enhanced cardiogenesis in animal models. Nevertheless, results in clinical trials remain unsatisfactory and determining a suitable cell population that is easily harvested and improves cardiac repair is challenging.

In previous research, our lab isolated human peripheral blood mononuclear clones (PBMCs) bearing cardiac mesodermal markers, e.g. c-kit, Islet-1 or Flk-1. Potentially, these cells can differentiate within the cardiac lineage to mature cardiomyocytes and participate in heart repair.

We sought to establish an *in vitro* cardioinstructive differentiation protocol to derive cardiomyocytes from our PBMC population. For this, we screened three differentiation protocols, i.e. Keller, Smith and He protocols. To follow cell differentiation RT-qPCR and immunostainings were performed for relevant genes, e.g. Nkx2.5, GATA4, Troponin T (cTNT), β-Myosin heavy chain (β-MHC) and α-actinin. Results for mRNA expression showed that at least 75% of PBMCs can derive to a cardiac precursor population (Nkx2.5$^+$/GATA4$^+$) following Keller and Smith protocol. In Smith protocol, 75% of PBMCs differentiated to immature cardiomyocytes (cTNT$^+$) of which 50% expressed both cTNT and β-MHC when co-cultured with neonatal mice ventricular myocytes. Immunofluoresence assay showed that PBMCs in both Keller and Smith protocol are Nkx2.5$^+$/α-actinin$^+$ demonstrating differentiation at the protein level.

Next we evaluated the safety, survival and integration of injected PBMCs in neonatal Gt(ROSA)^{26}-Tomato mice tissues *in vivo*. By staining PBMC with membrane marker PKH2GL, we followed cell homing within the animal tissues visualized by fluorescent microscopy on tissue sections.

In conclusion, we demonstrated that human PBMCs can differentiate, under certain conditions, into cardiac precursor cells or immature cardiomyocytes *in vitro*. These results make them an interesting and promising cell source for stem cell therapies in cardiac repair.

0090

Human cardiac progenitor cell seeded-collagen patches for cell therapy applied to right ventricular dysfunction: preliminary results in a large animal model

Catherine Rucker-Martin (1), Amir Hodzic (1), David Luu (2), Mathieu Coblence (3), Mohamed Ly (2), Emmanuel Le Bret (2), Elodie Gouadon (1), Marc Humbert (1), Philippe Jourdon (4), Michel Pucéat (4), Virginie Lambert (2)
(1) Centre Chirurgical Marie Lannelongue, INSERM U999, Le Plessis Robinson, France – (2) Centre Chirurgical Marie-Lannelongue, Cardiopathies Congénitales, Le Plessis Robinson, France – (3) Centre Chirurgical Marie Lannelongue, Laboratoire de Recherche Chiruirgical, Le Plessis Robinson, France – (4) Faculté de Médecine, INSERM UMR-S910, Team physiopathology of cardiac development, Marseille, France

Background: Cell therapy using intramyocardial injections of cardiac progenitors issued from human embryonic stem cells showed benefits on overloaded right ventricular (RV) tissue remodelling and arrhythmic susceptibility but this delivery mode failed to improve RV function. Our aim was to evaluate in a porcine model of overloaded RV dysfunction a new delivery mode of such therapy.

Methods: A combined overloaded RV dysfunction was obtained in piglets using a surgical procedure mimicking repaired tetralogy of Fallot. After 4 months, cell therapy was surgically administrated using 2 types of human NKX2.5+ cardiac progenitor cell-seeded collagen patches applied on the epicardium: QGel® and pressured-patches. Myocardial function was measured 1 month after transplantation by conductance catheter technique and echocardiography (standard and strain). The fate of progenitors was studied using antibodies directed against Ki67, CD31, actinin and Islet1.

Results: All pigs survived without any complication. Pressured-patches allowed human progenitors to migrate across the complete myocardium while QGel® patches restricted the cell migration to only a third of the myocardium. In both cases, progenitors differentiated toward the cardiac lineage assessed by Islet1 and actinin expression and maintained their proliferation capacity. Concerning RV function, only pressured-patches (N=3) tended to improve the contractility (Emax slope). By contrast, this parameter decreased in QGel® patches animals (N=2). Moreover, in 2 pressured-patch animals, standard echocardiographic functional parameters (FAC, TAPSE, s'wave) were maintained while 2D strain and strain rate values increased.

Conclusion: Cell therapy using seeded-patches was more conservative for engrafted cells than intramyocardial injections but only pressured-patches seemed to give benefits on overloaded RV function and contractility. These first promising results need to be checked at longer term.